Assessment of Heavy Metal Pollution in the Wetlands of Madhepura District Using Freshwater Fishes as Bioindicators

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SYNOPSIS

Proposed outline of the Research Work for Ph.D Degree in Zoology

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Title of Research:

Assessment of Heavy Metal Pollution in the Wetlands of Madhepura (Bhurkahi Chaur, Bairgachhi Chaur, Jorgama Wetland, Murliganj Wetland) District Using Freshwater Fishes as Bioindicators

1. Introduction

Wetlands are among the most productive freshwater ecosystems, providing ecological services such as nutrient cycling, aquifer recharge, biodiversity support, and fisheries productivity (Mitsch & Gosselink, 2000). The wetlands of Madhepura district—part of the dynamic Kosi basin—support significant aquatic biodiversity, including various species of fishes, plankton, mollusks, amphibians, and aquatic plants.

However, these wetlands are increasingly threatened by **heavy metal pollution**, primarily due to agricultural runoff, soil erosion, domestic waste, vehicular emissions, brick-kiln activities, small-scale industries, and atmospheric deposition (Sharma & Agrawal, 2005). Heavy metals such as **Pb, Cd, Cr, Ni, Cu, Zn, As, and Hg** are of high concern due to their toxicity, persistence, and bioaccumulative nature (Duffus, 2002).

Fishes are widely recognized as effective **bioindicators** of aquatic heavy metal contamination because they occupy various trophic levels and accumulate metals in their tissues over time (Authman et al., 2015). Metal exposure disturbs physiological, biochemical, hematological, and enzymatic functions in fishes (Canli & Atli, 2003). Chronic exposure can also cause oxidative stress, organ damage, reproductive impairment, and reduced survival.

Although studies from other regions of Bihar have reported metal contamination in rivers and ponds (Kumar et al., 2012), **no systematic study has been conducted on heavy metal levels in the wetlands of Madhepura district**, particularly using fish species as bioindicators. This research will fill this knowledge gap by assessing metals in water, sediment, and fish tissues, along with biomarker responses.

2. Origin of the Research Problem

The wetlands around *Bhurkahi Chaur, Bairgachhi Chaur, Murliganj Wetland,* and *Jorgama Wetland* receive agricultural runoff rich in fertilizers, pesticides, and eroded soil. Recent reports and informal surveys indicate deteriorating wetland health and declining fish diversity, similar to observations made in other agricultural landscapes (Jain et al., 2010).

Heavy metals accumulate in fish tissues such as gills, liver, kidney, and muscle (Rashed, 2001). These accumulations reflect environmental exposure and contamination levels. Farmers and fishermen in Madhepura have reported reduced fish abundance, increased mortality, and visible lesions—symptoms consistent with metal toxicity described by Al-Yousuf et al. (2000).

Absence of scientific monitoring and baseline ecological assessments necessitates a **comprehensive biomonitoring study**. This research aims to determine metal levels, assess biological stress responses, and identify sensitive bioindicator fish species.

3. Objectives of the Study

1. To estimate the concentration of heavy metals (Pb, Cd, Cr, Ni, Cu, Zn, As, Hg) in water and sediment of selected wetlands of Madhepura.
2. To determine heavy metal accumulation in tissues (gills, liver, kidney, muscle) of freshwater fish species (*Cirrhinus reba, Channa punctata, Labeo rohita*, etc.).
3. To analyze hematological parameters (RBC, WBC, Hb, PCV, blood indices) in fish collected from polluted vs. less-polluted wetlands.
4. To estimate biochemical and enzymatic markers (protein, glucose, glycogen, LDH, SDH, ATPase, AChE, antioxidant enzymes).
5. To investigate histopathological alterations in gills, liver, and kidney of collected fishes.
6. To identify suitable freshwater fish species as reliable bioindicators of metal pollution.
7. To correlate biological responses with heavy metal concentration through statistical modelling.

4. Hypothesis

It is hypothesized that:

- Wetlands receiving higher agricultural runoff will exhibit elevated concentrations of heavy metals in water, sediment, and fish tissues.
- Fishes from polluted sites will show significant deviations in hematological, biochemical, enzymatic, and histopathological parameters (Canli & Atli, 2003).
- Certain indigenous fish species will prove to be effective and sensitive bioindicators of metal contamination.

Thus, heavy metal contamination is expected to impair fish health and wetland biodiversity in Madhepura.

5. Methodology

A. Study Area Selection

Wetlands will be selected based on agricultural influence, anthropogenic load, and hydrological characteristics:

- Bhurkahi Chaur
- Bairgachhi Chaur
- Jorgama Wetland
- Murliganj Wetland

B. Sampling of Water and Sediment

Monthly sampling for one–two years.

Water quality parameters:
Temperature, pH, EC, DO, BOD, COD, TDS, alkalinity, hardness, nitrates, phosphates (APHA, 2012).

Heavy Metal Analysis:
Pb, Cd, Cr, Ni, Cu, Zn, As, Hg using AAS/ICP–MS (Grasshoff et al., 1999).

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C. Collection and Analysis of Fish

Selected species: *Cirrhinus reba, Channa punctata, Labeo rohita,* etc.

Dissection and tissue collection for:

- Gills
- Liver
- Kidney
- Muscle

D. Bioaccumulation Studies

Acid digestion of tissues using standard nitric-perchloric acid method followed by AAS/ICP–MS estimation. Bioaccumulation Factor (BAF) and Bioconcentration Factor (BCF) will be computed.

E. Hematological Studies

Using standard methods described by Blaxhall & Daisley (1973):

- RBC, WBC
- Hemoglobin (Hb)
- Hematocrit (PCV)
- MCV, MCH, MCHC

F. Biochemical and Enzymatic Analysis

Based on Lowry et al. (1951), Bergmeyer (1974), and standard protocols:

- Total protein
- Free amino acids
- Glucose and glycogen
- LDH, SDH
- ATPase, AChE
- Antioxidant enzymes: SOD (Marklund & Marklund, 1974), CAT (Aebi, 1984)

G. Histopathology

Gills, liver, and kidney will be processed following standard histological methods (Humason, 1979).

Slides will be examined for lesions, necrosis, vacuolation, lamellar fusion, and cellular degeneration.

H. Data Analysis

- One-way ANOVA
- Correlation and regression analysis
- Principal Component Analysis (PCA)
- Cluster Analysis for similarity among sites (Using SPSS/R software.)

6. Proposed Chapters of the Thesis

Chapter	Title
Chapter 1	Introduction
Chapter 2	Review of Literature
Chapter 3	Purpose and Hypothesis
Chapter 4	Materials and Methodology
Chapter 5	Results and Discussion
Chapter 6	Summary and Conclusion

7. Expected Outcomes

- Identification of priority wetlands with elevated heavy metal contamination.
- Determination of heavy metal accumulation in fish tissues and associated health risks.

- Establishment of hematological, biochemical, enzymatic, and histopathological biomarkers.
- Identification of effective fish bioindicator species for Madhepura wetlands.
- Contribution to environmental management, wetland conservation, and public health awareness.
- Development of a long-term biomonitoring framework for the district.

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